JOURNAL OF BACTERIOLOGY, Feb. 1988, p. 1021–1025
0021-9193/88/021021-05$02.00/0
Copyright © 1988, American Society for Microbiology
Vol. 170, No. 2

Cloning of the *Escherichia coli* K-12 *hemB* Gene

JIAN-MING LI,[1] H. UMANOFF,[1] R. PROENCA,[2] C. S. RUSSELL,[3] AND SHARON D. COSLOY[2]*

Departments of Biochemistry,[1] Biology,[2] and Chemistry,[3] City Collge, City University of New York, New York, New York 10031

Received 14 August 1987/Accepted 26 October 1987

An *Escherichia coli* heme-requiring, heme-permeable mutant had no detectable 5-aminolevulinate dehydratase or porphobilinogen deaminase activities. The gene which complemented this mutation was cloned to a high-copy-number plasmid, and porphobilinogen deaminase activity was restored to normal levels, but the synthesis of 5-aminolevulinate dehydratase increased 20- to 30-fold. A maxicell procedure confirmed that the gene cloned was *hemB*.

The heme molecule is the prosthetic group of the hemoglobins, cytochromes, catalases, and peroxidases. Modified tetrapyrroles are the active moieties of the chlorophylls and vitamin B_{12}. The heme biosynthetic pathway consists of eight enzymatically catalyzed steps.

In *Escherichia coli*, the heme genes encoding these enzymes are widely scattered on the genome (2, 3, 15, 26). Two of the heme genes in *E. coli* have been cloned and sequenced: *hemC* (porphobilinogen deaminase [PBG-D]) (39) and *hemD* (urogen cosynthase) (31). We report here the cloning of *hemB* (5-aminolevulinate dehydratase or PBG synthase [ALA-D]) from *E. coli*. ALA-D catalyzes the dimerization of ALA to form the monopyrrole PBG. The latter subsequently tetramerizes and leads to the various tetrapyrrole intermediates in the pathway to heme. ALA-D has been isolated and characterized from various sources (12), including human (1), duck (35), and chicken erythrocytes (13), bovine liver (6, 14, 43, 44), mouse (7, 10), guinea pig (40), plants (18, 20, 23, 36, 37, 38), and bacteria (*Rhodopseudomonas spheroides* [5, 21], *Rhodopseudomonas capsulata* [22], and *Mycobacterium phlei* [45]). While the enzymes from all sources appear to be octamers of subunits of 31 to 35 kilodaltons (kDa), there are significant differences in susceptibility to chelating agents and activation by cations. The human ALA-D gene has been cloned and sequenced by Bishop et al. (4) and Wetmur et al. (41, 42). Cloning of the *E. coli hemB* gene provides an opportunity to prepare enough DNA to sequence the control and structural regions and to prepare comparatively large amounts of the enzyme for characterization and study.

The strains used in this study are listed in Table 1. A *hemB* mutant was isolated by using the technique of neomycin resistance to select for respiratory mutants (29, 30, 32). Strain C600 was plated out on LB medium (34) supplemented with 50 μg of neomycin per ml. The 800 microcolonies that arose spontaneously were screened for catalase activity and ALA dependence. The mutant chosen (RP522) was catalase negative, was unable to grow well on medium supplemented with ALA, and did not accumulate porphyrins in its growth medium.

Since the mutant was a presumptive heme mutant and could not respire, it grew very poorly. It could not be supplemented with heme since *E. coli* is impermeable to heme. Therefore, RP522 was mutagenized to heme permeability with *N*-methyl-*N*-nitro-*N*′-nitrosoguanidine. After mutagenesis, cells were spread on hemin-supplemented minimal medium, and medium- to large-sized colonies were purified. One such strain chosen for further study (RP523) required 10 μg of hemin per ml for growth on both minimal medium (19) and rich peptone media, such as LB. In addition, the strain was sensitive to 100 μg of actinomycin per ml, whereas C600, the original parent, is resistant to this concentration of the drug. Thus, it is likely that RP523 carries a defect which alters permeability and allows it to utilize hemin from the medium and renders it sensitive to actinomycin.

The *hem* mutation was mapped by using an F′ kit. The mutation was complemented with F′254 to yield heme prototrophs but not with F′128 or F′152 and therefore lies within the 8- to 11-min map region containing *hemB* (ALA-D) and *hemH* (ferrochelatase) (3). Enzyme assays were carried out. All strains were grown to saturation in LB medium. Strains harboring plasmids were grown in medium supplemented with 50 μg of ampicillin per ml. Cells were washed as follows: for the ALA-D assay, 50 mM sodium phosphate buffer (pH 6.4); for the PBG-D assay, 0.1 M Tris hydrochloride (pH 8.2); for the ferrochelatase assay, 47.6 μM potassium phosphate buffer (pH 7.8). All cells were suspended in 1 ml of the respective buffer and sonicated on ice for a total of 90 s (with a 10-s interval between each 10-s sonication). ALA-D activity was determined by using the procedure of Sassa (33) scaled up four times with the following modifications. (i) The pH of the sodium phosphate was 6.4 instead of 5.8. (ii) The assay mixture contained 200 μl of assay mix plus 20 μl of the extract and 80 μl of sodium phosphate buffer. (iii) The incubation time was 1.5 h. Activity is reported as A_{553} per milligram of protein. PBG-D activity was determined by the following procedure. A 10-μl sample of the extract, 10 μl of PBG (concentration, 1 mg/ml), and 80 μl of 0.1 M Tris hydrochloride (pH 8.2) were incubated at 37°C for 2 h in the dark; 1 ml of 1 M HCl was then added, and the precipitate was removed by centrifugation. The spectrum of the supernatant from 450 to 350 nm was recorded. Activity is reported as A_{405} per milligram of protein. The procedure of Porra and Jones (25) was followed for the ferrochelatase assay. Activity is reported as heme produced per milligram of protein (nanomoles per milligram). Protein concentration was determined by the Bio-Rad method (Bio-Rad Laboratories, Richmond, Calif.).

The mutant synthesized neither ALA-D (*hemB*) nor PBG-D (*hemC*) but did synthesize a substantial amount of ferrochelatase (Table 2).

To determine whether the isolated mutant carried a mu-

* Corresponding author.

TABLE 1. Strains, plasmids, and phage used in this study

Strain, plasmid, or phage	Relevant genotype or phenotype[a]	Source (reference)[b]
Strains		
C600		CGSC
RP522	C600, but *hemB*	This lab
RP523	RP522, but heme permeable	This lab
HU1000	RP523(pSE103)	This lab
HU1016	HU1000(phHU16)	This lab
CSR603	*phr-1 recA uvrA6*	D. Calhoun (28)
JL1002	RP523(pJL2)	This lab
JL1102	C600(pJL2)	This lab
JM101	Δ(*lac-proAB*) (F′ *traD36 proAB lacI*^{q} *lacZ*Δ*M15*)	U.S. Biochemical
JC7623	*recB recC sbcB*	A. J. Clark (17)
Plasmids or phage		
λ SE6	*E. coli* genomic library	ATCC (11)
pSE103	λ *c*I857 Kan^r	ATCC (11)
pTZ18U		U.S. Biochemical
phHU16	Hem$^+$ (from λ SE6)	This lab
pJL2	Hem$^+$ (from pTZ18U)	This lab
F′ kit		CGSC

[a] Other genetic markers are C600, *thr-1 leuB6 thi-1 lacY1 tonA21 supE44* λ$^-$ F$^-$; CSR603, *thr-1 leuB6 proA2 argE3 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 rpl-31 tsx-33 supE44* λ$^-$ F$^-$; JM101, *supE thi-1*; JC7623, *his-4 ara-14 thr-1 thi-1 leuB6 proA2 argE3 rpl-31 lacY-1 galK2 xyl-5 tsx-33 sup-37*(Am) λ$^-$ F$^-$.

[b] CGSC, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.; ATCC, American Type Culture Collection, Rockville, Md.; U.S. Biochemical, U.S. Biochemical Corp., Cleveland, Ohio.

tant allele for *hemB* or for some heme pathway regulatory gene, the gene that complemented the mutation was cloned. The cloning strategy is presented in Fig. 1. An *E. coli* genomic library in λ SE6 (11) was introduced by bacteriophage infection into HU1000, the *hem* mutant carrying a cloned temperature-sensitive allele of the gene encoding the λ phage repressor protein. Under these conditions, this phage behaves as a phasmid, a single-copy plasmid (11). Hem$^+$ colonies were selected on minimal medium lacking hemin. A total of 20 such colonies were purified, and phasmid DNA was isolated (9) from 13 of them. The phasmid DNA was prepared from the phage by the method of Kaslow (16) with the following modification. The DNA was precipitated at 80°C for 30 min and centrifuged at 10,000 × *g* at 4°C for 25 min. The DNAs were amplified by transfection into JC7623 (8), which allows the DNA to be incorporated into phage particles, and subsequent growth of phage lysates. The phage lysates (19) from many of the Hem$^+$ transductants had low titers (<106 PFU/ml), but three of the transductants produced phages that gave relatively high titers upon growth (10^8 to 10^{10} PFU/ml). One of these transductants, HU1016, was chosen for recloning of its insert.

The phasmid (phHU16) isolated from HU1016 contained a 17-kilobase (kb) insert of *E. coli* genomic DNA. Since we believed that we had complemented a *hemB* mutation with the phasmid, and the *lac* operon has been mapped close to *hemB* (2, 3, 15, 26), we tested the ability of HU1016 to grow on medium containing lactose as a sole carbon source to determine whether the phasmid also carried the *lac* locus which would complement the *lacY* mutant allele of HU1016. The strain could not grow on medium containing lactose as a sole carbon source. A second phasmid carrying *hemB* was also tested for the *lac* locus. It, too, did not complement the *lacY* mutation of HU1016.

The Hem$^+$ transductant strain was assessed for its ability to synthesize heme pathway enzymes. The transductant, which carries the single-copy phasmid DNA, produced wild-type levels of ALA-D, PBG-D, and ferrochelatase. Thus, the insert carried by the phasmid restored the enzyme activities lost by the mutant.

The phasmid DNA (16) was partially digested with *Sau*3A, and the DNA was ligated with plasmid pTZ18U at its *Bam*HI site, which is located in a *lacZ* gene. Resulting plasmids were transformed into JM101, and white colonies were selected on medium with X-Gal (5-bromo-4-chloro-3-indolyl-β-galactopyranoside). DNA was prepared from purified white colonies and used to transform RP523 to heme prototrophy.

One such plasmid, carrying the gene for heme prototrophy, was found to have a 7-kb insert (pJL1). This plasmid was double digested with *Bam*HI and *Eco*RI, and the fragment was recloned into pTZ18U (pJL2). A 2.85-kb fragment contained the gene. Digestion of this fragment with *Sal*I or *Hin*dIII abolished gene activity. The fragment was not cut by the following enzymes: *Eco*RI, *Bgl*II, *Bam*HI, *Sac*I, *Xba*I, *Kpn*I, and *Mbo*I. A partial restriction map of the fragment is shown in Fig. 1. Strains carrying the plasmid produced about 20 to 30 times the wild-type levels of ALA-D and normal levels of PBG-D and ferrochelatase (Table 2).

A maxicell protocol (27) was used to determine which genes were cloned on pJL2. The plasmid was transformed into CSR603, and the maxicell procedure was carried out. CSR603 was transformed with pTZ18U and pJL2 (19). The method of Sancar et al. (27) was followed with the following modifications. (i) CSR603 and its derivatives were grown in

TABLE 2. Activities of heme biosynthetic enzymes in wild-type strains and strains constructed in this study

Strain	Activity of[a]:		
	ALA-D	PBG-D	Ferrochelatase
RP523	0	0	0.319
HU1016	0.41	0.21	0.630
C600	0.41	0.37	0.927
JL1002	10.05	0.33	0.670
JL1102	8.30	0.32	0.606

[a] For units of activity, see text.

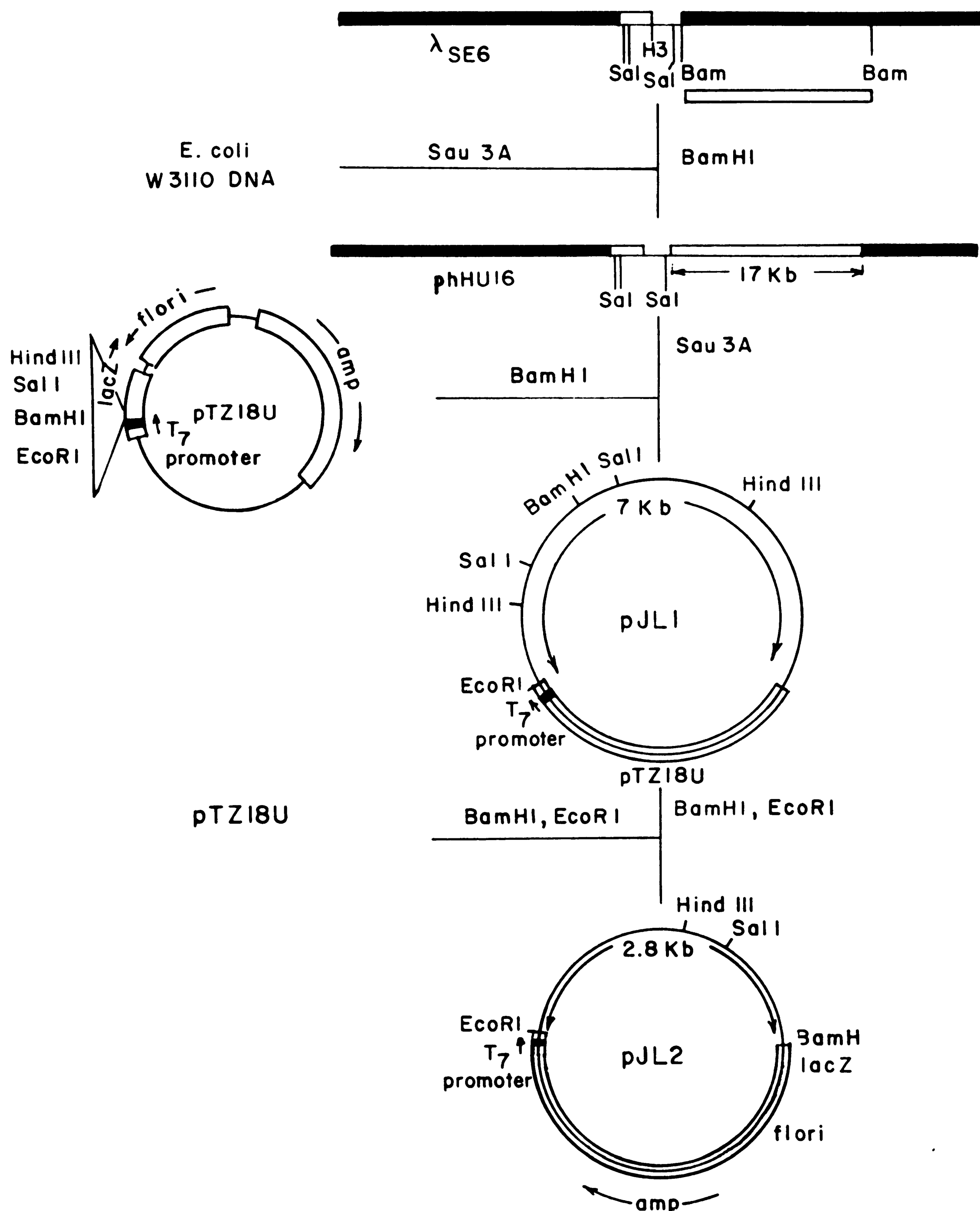

FIG. 1. Strategy for cloning *hemB*.

M9 (19) with 1% Casamino Acids (Difco Laboratories, Detroit, Mich.) and supplements of threonine, leucine, proline, and arginine at 50 μg/ml each and thiamine at 5 μg/ml. (ii) Cells were irradiated at a density of 2×10^8 cells per ml in a glass petri dish, with rotary shaking for 6 s at a distance of 60 cm from a Westinghouse Sterillamp G15T8. (iii) Cells were grown at 37°C for 2 h after irradiation, and D-cycloserine was then added at a final concentration of 4 μg/ml (24). Incubation continued for 14 h. (iv) After being washed, cells were suspended in buffer depending on the enzyme assay to be performed and sonicated if enzyme assays and nondenaturing electrophoresis were to be performed. After UV irradiation and subsequent growth of the cells, extracts were prepared. These extracts were assayed for enzymatic activity and analyzed on polyacrylamide gels. The extracts had 100 times the normal ALA-D activity of CSR603(pTZ18U). pJL2 directed the production of two proteins which have subunit sizes of 28 and 38 kDa, as determined when they were resolved on sodium dodecyl sulfate-polyacrylamide gels (Fig. 2). The control plasmid pTZ18U directed only the synthesis of a 28-kDa protein, which is the β-lactamase synthesized by the *amp* gene on the plasmid. Nondenaturing

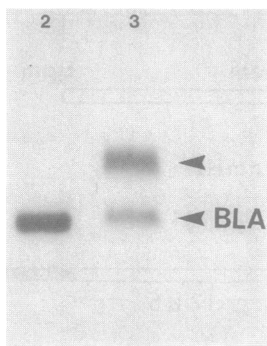

FIG. 2. Autoradiogram of proteins produced in the presence of [^{35}S]methionine in the maxicell procedure (19, 24, 27). The proteins were analyzed on sodium dodecyl sulfate–10% acrylamide disc gels. Lanes: 1, CSR603; 2, CSR603(pTZ18U); 3, CSR603(pJL2). Lane 1 was blank. Coomassie blue staining of these samples and standards showed that the band in lane 2 had a molecular size of 28 kDa (β-lactamase [BLA]) and bands in lane 3 had molecular sizes of 28 (β-lactamase) and 38 (ALA subunit) kDa, respectively.

gels revealed three protein bands in addition to that of β-lactamase (Fig. 3). These three bands had ALA-D activity and probably correspond to different aggregations of subunits. PBG-D activity was not associated with the protein on the gel. Thus, the *hemB* gene, and not some regulatory gene, was cloned.

In summary, we have cloned the *hemB* gene of *E. coli* by complementing a strain which harbors a mutant allele for *hemB*. Strains which carry the cloned plasmid produced 20 to 30 times more ALA-D than wild-type strains did. The maxicell procedure demonstrates that the plasmid encodes one protein other than β-lactamase. This protein has a subunit size of 38 kDa and has ALA-D activity in at least two aggregated forms.

The mutation mapped to the general region where *hemB* is found on the *E. coli* map (2, 15, 26). However, we could not confirm its precise location as tightly linked to the *lac* locus. According to the published map, *lac* is located less than 0.25 min away from *hemB* (2). The original phasmid from which we subcloned the *hemB* gene contained a 17-kb insert. Since 1 map min contains 45 kb of DNA (2), it was possible that the phasmid would also carry the *lac* locus. This phasmid and a

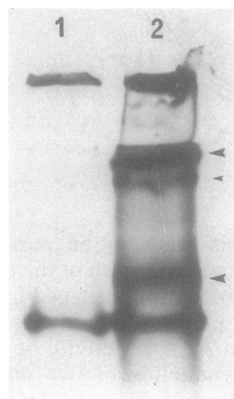

FIG. 3. Autoradiogram of proteins produced in maxicell procedure in the presence of [^{35}S]methionine. The proteins were analyzed on a nondenaturing 10% polyacrylamide disc gel. Lanes: 1, CSR603(pTZ18U); 2, CSR603(pJL2). The enzyme assay showed that the bands indicated by arrows had ALA-D activity.

second one tested did not complement a *lacY* mutation. It is possible that the phasmids only carried *E. coli* genomic material downstream from *lac* or that the distance between *hemB* and *lac* is actually greater than that found previously. We are testing other Hem$^+$ phasmids to determine whether they carry the *lac* locus.

RP523 is deficient in both ALA-D and PBG-D activities. The cloned plasmid which complements this strain overproduces ALA-D but restores PBG-D activity to normal levels. Since, as seen from the maxicell procedure, the plasmid produces only one insert-associated protein and this protein is ALA-D, the gene cloned must be the structural gene for ALA-D and not some regulatory locus. However, it is obvious that the *hemB* mutant allele must be affecting the regulation of *hemC*, even though it maps a substantial distance away. It is possible that the product of ALA-D action, PBG, is controlling the expression of PBG-D. This contention is supported by our previous observations (H. Umanoff, R. Proenca, C. S. Russell, and S. D. Cosloy, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, H129, p. 161) that when grown on heme, heme-permeable strains which are wild type or deficient in ALA synthase (*hemA*) have no PBG-D activity unless ALA is added to the growth medium. Presumably, the ALA is converted to endogenous PBG. RP523, which has an absolute heme requirement for growth and cannot make PBG, produces no PBG-D activity in the presence of ALA. If PBG does in fact control the synthesis of PBG-D, then RP523 should synthesize PBG-D when grown on medium supplemented with PBG. However, we found that RP523 did not produce PBG-D in the presence of PBG. We believe that PBG does not permeate the cells, as evidenced by the inability of *hemA* and *hemB* mutants to grow on medium supplemented with PBG.

We thank D. Calhoun for providing CSR603 and B. Bachmann for providing the F′ strain kit. We also thank P. Margolin for helpful discussions.

This work was supported in part by Public Health Service grants RR08168 (MBRS) and RR03060 (RCMI) from the National Institutes of Health.

LITERATURE CITED

1. **Anderson, P. M., and R. J. Desnick.** 1979. Purification and properties of Δ-aminolevulinate dehydrase from human erythrocytes. J. Biol. Chem. **254:**6924–6930.
2. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47:**180–230.
3. **Bachmann, B. J., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K-12, edition 6. Microbiol. Rev. **44:**1–56.
4. **Bishop, J. R., P. J. Cohen, S. H. Boyer, A. N. Noyes, and L. P. Frelin.** 1986. Isolation of a rat liver Δ-aminolevulinate dehydrase (ALAD) cDNA clone: evidence for unequal ALAD gene dosage among inbred mouse strains. Proc. Natl. Acad. Sci. USA **83:**5568–5572.
5. **Burnham, B. F., and J. Lascelles.** 1963. Control of porphyrin biosynthesis through a negative-feedback mechanism. Biochem. J. **87:**462–472.
6. **Cheh, A., and J. B. Neilands.** 1973. Zinc, an essential metal ion for beef liver Δ-aminolevulinate dehydratase. Biochem. Biophys. Res. Commun. **55:**1060–1063.
7. **Coleman, D. L.** 1966. Purification and properties of Δ-aminolevulinate dehydratase from tissues of two strains of mice. J. Biol. Chem. **241:**5511–5517.
8. **Davis, R. W., D. Botstein, and J. R. Roth (ed.).** 1980. Advanced bacterial genetics, p. 140–141. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **de Bruijn, F. J., and F. M. Ausubel.** 1981. The cloning and transposon mutagenesis of the *glnA* region of *Klebsiella pneumoniae*: identification of *glnR*, a gene involved in the regulation of the *nif* and *hut* operons. Mol. Gen. Genet. **183:**289–297.

10. **Doyle, D., and R. T. Schimke.** 1969. The genetic and developmental regulation of hepatic Δ-aminolevulinate dehydratase in mice. J. Biol. Chem. **244**:5449–5459.
11. **Elledge, S. J., and G. C. Walker.** 1985. Phasmid vectors for identification of genes by complementation of *Escherichia coli* mutants. J. Bacteriol. **162**:777–783.
12. **Gibson, K. D., A. Neuberger, and J. J. Scott.** 1955. The purification and properties of Δ-aminolaevulic acid dehydrase. Biochem. J. **61**:618–629.
13. **Granick, S.** 1954. Enzymatic conversion of Δ-aminolevulinic acid to porphobilinogen. Science **120**:1105–1106.
14. **Gurba, P. E., R. E. Sennett, and R. Kobes.** 1972. Studies on the mechanism of action of Δ-aminolevulinate dehydratase from bovine and rat liver. Arch. Biochem. Biophys. **150**:130–136.
15. **Hadley, R. G., M. Hu, M. Timmons, K. Yun, and R. C. Deonier.** 1983. A partial restriction map of the *proA-purE* region of the *E. coli* K12 chromosome. Gene **22**:281–287.
16. **Kaslow, D. C.** 1986. A rapid biochemical method for purifying lambda DNA from phage lysates. Nucleic Acids Res. **14**:6767.
17. **Kushner, S. R., H. Nagaishi, A. Templin, and A. J. Clark.** 1971. Genetic recombination in *Escherichia coli*: the role of exonuclease I. Proc. Natl. Acad. Sci. USA **68**:824–827.
18. **Liedgens, W., C. Lutz, and H. A. W. Schneider.** 1983. Molecular properties of 5-aminolevulinic acid dehydratase from *Spinacia oleraiea*. Eur. J. Biochem. **135**:75–79.
19. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual, p. 440. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Maralihalli, G. B., S. R. Rao, and A. S. Bhagwat.** 1985. Histidine residues at the active site of maize Δ-aminolevulinic acid dehydratase. Phytochemistry **24**:2533–2536.
21. **Nandi, D. L., and D. Shemin.** 1968. Δ-Aminolevulinic acid dehydratase of *Rhodopseudomonas spheroides*. J. Biol. Chem. **243**:1231–1235.
22. **Nandi, D. L., and D. Shemin.** 1973. Δ-Aminolevulinic acid dehydratase of *Rhodopseudomonas capsulata*. Arch. Biochem. Biophys. **158**:305–311.
23. **Nandi, D. L., and E. R. Waygood.** 1967. Biosynthesis of porphyrins in wheat leaves. II. Δ-Aminolevulinate hydro-lyase. Can. J. Biochem. **45**:327–336.
24. **Perbal, B.** 1984. A practical guide to molecular cloning, p. 529–532. John Wiley & Sons, Inc., New York.
25. **Porra, R. J., and O. T. G. Jones.** 1963. Studies on ferrochelatase. I. Assay and properties of ferrochelatase from a pig liver mitochondrial extract. Biochem. J. **87**:181–185.
26. **Powell, K. A., R. Cox, M. McConville, and H. P. Charles.** 1973. Mutations affecting porphyrin biosynthesis in *Escherichia coli*. Enzyme **16**:65–73.
27. **Sancar, A., A. M. Hack, and W. D. Rupp.** 1979. Simple method for identification of plasmid-coded proteins. J. Bacteriol. **137**:692–693.
28. **Sancar, A., and W. D. Rupp.** 1978. Correction of the map location for the *phr* gene in *Escherichia coli* K12. Mutat. Res. **51**:139–143.
29. **Sasarman, A., P. Chartrand, M. Lavoie, D. Tardif, R. Proschek, and C. Lapointe.** 1979. Mapping of a new *hem* gene in *Escherichia coli*. J. Gen. Microbiol. **113**:297–303.
30. **Sasarman, A., and T. Horodniceau.** 1963. Utilisation de la neomycin pour la selection des variantes naines de salmonella. Arch. Roum. Pathol. Exp. Microbiol. **22**:1101–1110.
31. **Sasarman, A., A. Nepveu, Y. Echelard, J. Dymetryszyn, M. Drolet, and G. Goyer.** 1987. Molecular cloning and sequencing of the *hemD* gene of *Escherichia coli* K-12 and preliminary data on the Uro operon. J. Bacteriol. **169**:4257–4262.
32. **Sasarman, A., K. E. Sanderson, M. Surdeanu, and S. Sonea.** 1970. Hemin-deficient mutants of *Salmonella typhimurium*. J. Bacteriol. **102**:531–536.
33. **Sassa, S.** 1982. Δ-Aminolevulinic acid dehydratase assay. Enzyme **28**:133–145.
34. **Schleif, R. C., and P. C. Wensink.** 1981. Practical methods in molecular biology, p. 196–197. Springer-Verlag, New York.
35. **Schmid, R., and D. Shemin.** 1955. The enzymatic formation of porphobilinogen from Δ-aminolevulinic acid and its conversion to protoporphyrin. J. Am. Chem. Soc. **77**:506–507.
36. **Shetty, A. S., and G. W. Miller.** 1969. Δ-Aminolevulinate dehydratase from ox liver and tobacco leaves. Biochim. Biophys. Acta **185**:458–460.
37. **Shibata, H., and H. Ochiai.** 1976. Purification and properties of Δ-aminolevulinic acid dehydratase from radish cotyledons. Plant Cell Physiol. **18**:421–429.
38. **Stella, A. M., and A. M. del C. Batlle.** 1978. Porphyrin biosynthesis. Immobilized enzymes and ligands. VIII. Studies on the purification of Δ-amino-levulinate dehydratase from *Euglena gracilis*. Plant Sci. Lett. **11**:87–92.
39. **Thomas, S. D., and P. M. Jordan.** 1986. Nucleotide sequence of the *hemC* locus encoding porphobilinogen deaminase of *Escherichia coli* K12. Nucleic Acids Res. **14**:6215–6226.
40. **Weissberg, J. B., and P. E. Voytek.** 1974. Liver and red cell porphobilinogen synthase in the adult and fetal guinea pig. Biochim. Biophys. Acta **364**:304–319.
41. **Wetmur, J., D. F. Bishop, L. Ostasiewicz, and R. J. Desnick.** 1986. Molecular cloning of a cDNA for human Δ-aminolevulinic acid dehydratase. Gene **43**:123–130.
42. **Wetmur, J. G., D. F. Bishop, C. Cantelmo, and R. G. Desnick.** 1986. Human Δ-aminolevulinic acid dehydratase: nucleotide sequence of a full-length cDNA clone. Proc. Natl. Acad. Sci. USA **83**:7703–7707.
43. **Wilson, E. L., P. E. Burger, and E. B. Dowdie.** 1972. Beef liver Δ-aminolevulinic acid dehydratase. Eur. J. Biochem. **29**:563–571.
44. **Wu, W. H., D. Shemin, K. E. Richards, and R. C. Williams.** 1974. The quaternary structure of Δ-aminolevulinic acid dehydratase from bovine liver. Proc. Natl. Acad. Sci. USA **71**:1767–1770.
45. **Yamasaki, H., and T. Moriyama.** 1971. Δ-Aminolevulinic acid dehydratase of *Mycobacterium phlei*. Biochim. Biophys. Acta **227**:698–705.